Original Research Paper

Volume-7 | Issue-12 | December-2017 | ISSN - 2249-555X | IF : 4.894 | IC Value : 86.18

Microbiology

BACTERIAL PROFILE OF CHRONIC ULCERS-A STUDY ON NON-DIABETIC PATIENTS

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ABSTRACT

Back Ground: Chronic foot and leg ulcers are very common in the general population which restricts the individual from a productive social and normal personal life. Most of the time patient needs multiple hospital admissions, treatment with multiple courses of antibiotics, surgical treatment ranging from slough cutting to amputation depending upon the severity and underlying cause. Patients with chronic ulcers come under two groups, diabetics and non diabetics.

Objectives: To study the bacterial spectrum in non-diabetic and also to determine the antibiotic sensitivity pattern in this group.

Methods: A descriptive study was conducted in 87 non-diabetic patients admitted in the general surgery wards of Government Medical College Kottayam, with leg and foot ulcers of more than 6 weeks duration. Samples were sent to Microbiology laboratory for culture and sensitivity. Bacteria were identified by culture, microscopy and antibiotic sensitivity was done by conventional methods.

Results: All the samples were culture positive and polymicrobial non diabetics. *Staphylococci* (54.14%) was the most commonly identified pathogen in non-diabetics. Gram positive cocci showed high sensitivity to Vancomycin,Cloxacillin and Amikacin but shows high resistance to Penicillin, Eythromycin and 1st generation Cephalosporins. Gram negative bacilli were highly sensitive to Imipenem, Meropenem, Piperacillin-Tazobactum, Cefoperazone sulbactam and Amikacin and they showed high resistance to Ampicillin and Ciprofloxacin.

Conclusions: It is always better to do culture and sensitivity before starting antibiotics in patients with chronic ulcers.

KEYWORDS : Non-diabetic ulcer, Staphylococci, Pseudomonas, Antibiotic sensitivity

INTRODUCTION

An ulcer is defined as discontinuity of an epithelial surface. It is characterized by progressive destruction of the surface epithelium and a granulating base[1] .A chronic ulcer is defined as an ulcer more than 6 weeks duration[2].There are only few Indian studies on the epidemiology of chronic wounds. One study estimated the prevalence at 4.5 per 1000 population [3]. In the Western world, leg ulcers are mainly caused by venous insufficiency, arterial insufficiency, neuropathy, diabetes or a combination of these factors. The study from India shows that etiology of chronic wounds included systemic conditions such as diabetes, atherosclerosis, tuberculosis, leprosy etc. The study report stated that inappropriate treatment of acute traumatic wounds was the most common cause of the chronic wounds [4]. Chronic diabetic ulcers are usually polymicrobial and the bacteria isolated from both diabetic and non diabetic were the same even though they vary in their frequency rate of infection (5). Bacterial population in chronic ulcers do not differ significantly in many studies even though the positive culture report varies in these groups and most of them are resistant to usually using and easily available antibiotics. This study was to describe the bacterial spectrum causing chronic foot and leg ulcers in non diabetic individuals and to describe their antibiotic sensitivity pattern. Both diabetic and non diabetic had the history of multiple trials of antibiotics and hospitalization. Seven species of bacteria were isolated from both diabetic and non diabetic groups, so the spectrum of bacteria causing infection in chronic foot and leg ulcers in both diabetic and non diabetic were the same; even though the prevalence of infection was different.

MATERIALS AND METHODS

This descriptive study was done in non- diabetic ulcer sample send to Microbiology department of Government Medical College Kottayam from July 2014 to June 2015. Samples were collected from all patients admitted in the in the wards of General Surgery, with chronic foot and leg ulcers of more than 6 weeks duration during the study period. Out of a total of 234 samples, 87 were selected into the study.

Inclusion criteria: Patients with leg and foot ulcers of more than 6 weeks

Exclusion criteria: Diabetic patients.

Sample collection

Culture specimens were obtained at the time of admission after local debridement of devitalized tissues. The ulcer wound was scrubbed thoroughly with sterile normal saline to remove superficial exudates. Sample collection was done using sterile cotton swabs. Two swabs were collected, one for gram –stain and the other for aerobic culture. Samples were taken immediately to the laboratory.

Microscopic examination

The type and relative number of microorganisms and pus cells were identified by direct gram stained smear of all the samples.

Identification procedures[6]

For aerobic culture, the specimens were inoculated on 5% Sheep Blood agar & MacConkey agar. Salt agar was used for the isolation of *Staphylococcus.* Glucose broth was also inoculated as back up broth. The inoculated plates and broth were then incubated at 37°C for 24 hours. The isolates were identified based on colony morphology, gram-staining results, motility, catalase test, oxidase test, coagulase test and biochemical tests using standard laboratory procedures [7,8] .

Anaerobic bacteria were not investigated owing to limited laboratory facilities. Anaerobes are rarely seen as single pathogen usually found in mixed flora.

Antibiotic sensitivity

It was done by Kirby-Bauer disc diffusion method. [9] Muller Hinton agar plates and commercially available Hi-media discs were used as per Clinical and Laboratory Standards Institute Guidelines.[10]

Disc concentration for antibiotics

Penicillin (10 IU), Ampicillin (10µg), First generation Cephalosporin (30µg), Erythromycin (15µg), Gentamicin ((10µg, 120 µg), Amikacin (30µg), Ciprofloxacin (5µg), Vancomycin (30µg), Linezolid (30µg), third generation cephalosporins, Piperacillin-tazobactam (100/10µg). Inoculum was standardized using 0.5 McFarland's opacity standard and reporting based on CLSI guidelines [10] .

RESULTS

During the study period a total of 87 samples were taken from non-diabetic patients. All the samples showed culture positivity of which 24.14% were polymicrobial.

Table 1. Over view of culture done

Category	No.of patients	percentage
Diabetic	147	62.8
Non-diabetic	87	37.2
Total	234	100

Table 2 Percentage of bacteria Cultured from non-Diabetic Patients

Bacteria	Percentage
Methicillin Sensitive Staphylococci(MSSA)	12.64%
Methicillin Resistant Staphylococcus aureus(MRSA)	32.18%
Coagulase negative Staphylococci (CoNS)	9.19%
Streptococci	0.34%
E.coli	19.84%
Psuedomonas	19.54%
Klebsiella spp.	13.79%
Acinetobacter	10.34%
Proteus	6.89%.

non- diabetics, *Staphylococci (54.01%)* was the most frequent bacteria isolated of which the *MRSA* constituted 32.18% and *MSSA* 12.64% and CoNS 9.19%.(11) Among gram negative isolates *E.coli (*19.84%), *Pseudomonas (*19.59%) *Klebsiella (*13.79%), *Proteus* (6.89%), *Acinetobacter (*10.34%), and *Streptococci (* 0.34%) were isolated.

Table 3.Antibiotic sensitivity of Staphylococci (% sensitivity)

Antibiotics	MSSA	MRSA	CoNS
Penicillin	0	0	12.50
Ampicillin	100	-	25
Cloxacillin	100	-	37.50
Gentamicin	0	17.85	50
Amikacin	80	67.85	87.5
1st generation Cephalosporin	100	14.28	25
Vancomycin	100	100	100
Erythromycin	10	0	-
Linezolid	60	3.57	25

Staphylococci showed good susceptibility to Cephalosporins, Vancomycin (100%) to aminoglycosides (90%) and high resistance to Erythromycin and Ampicillin. *MRSA* showed 100% sensitivity to Vancomycin and 67% to Amikacin, . It was highly resistant to all other antibiotics. Coagulase negative *Staphylococci* were highly susceptible to Vancomycin and also to Amikacin.

Streptococci - least commonly isolated bacteria in non diabetics was sensitive to commonly using antibiotics.

Gram negative bacilli isolated were *Esherichia coli*, *Pseudomonas*, *Klebsiella*, *Proteus* and *Acinetobacter.* These were highly sensitive to Imipenem, Meropenem, Piperacillin- tazobactum, Cefoperazone-sulbactam and Amikacin (80-100%) in both groups.

In the study by Mrs Smita Watwe et al. [11], *Staphylococcus aureus* was the single most common isolate, from 34 of 86 cases (40%). *Staphylococcus aureus* dominated the non-diabetic group as a single isolate from 16 cases (43.2%). Gram negative bacilli were isolated on 44 occasions (61%) from diabetics, but in non-diabetics it was limited to 43.2% only. Among the gram negative bacilli, *Proteus spp.* was common (19%) in diabetics and in non-diabetics *Pseudomonas spp.* was isolated in 16% cases.

In this study also in non diabetics Gram positive cocci were the most prevalent isolates.

Table 4 Antibiotic sensitivity of Gram negative bacteria

Isolates	A	G	AK	CIP	1st CEPH	CT	CP+S	P+T	MERO
Pseudomonas	-	29.40	47.05	35.29	-	11.36	11.36	58.82	58.82
Klebsiella	-	18.18	90.90	36.36	27.27	36.36	72.72	72.72	72.72
E.coli	0	29.41	82.35	35.29	17.64	23.52	47.05	64.70	70.15
Proteus	20	60	80	80	10	0	-	80	100
Acinetobacter	11.11	11.11	33.33	22.22	10	0	22.22	44.44	44.44

Methicillin Sensitive Staphylococcus [12, 13, 14)
Gram positive cocci arranged in grape like clusters. On blood agar golden yellow colonies are formed. On salt agar it produces creamy white opaque colonies. Pink colonies in MacConkey agar. Black colonies in Tellurite blood agar. Catalase and Coagulase test positive. It reduces Nitrate to nitrite. Methyl red (MR) test andVoges-Proskauer (VP) tests are positive. Fermentative utilization of glucose on Oxidation-Fermentation (O/F) media [8]. It ferments Mannitol and produces acid. It hydrolyses urea. DNAse test is positive[15]. Phosphatase test positive.

***Methicillin Resistant Staphylococcus aureus (MRSA)*[16]**
Methicillin-resistant *Staphylococcus aureus* (MRSA) is a bacteria that is resistant to many antibiotics. In the community, most MRSA infections are skin infections. In medical facilities, MRSA causes life-threatening blood stream infections, pneumonia and surgical site infections. These bacteria are typically resistant to most of beta-lactam and many non betalactam antibiotics.

Coagulase negative Staphylococci (*Staphylococcus epidermidis)* [16]
Gram positive cocci arranged in grape like clusters. White opaque colonies in salt agar. Catalase positive. Phosphatase test- positive. Slide and tube Coagulase negative. Bacitracin 0.04 units resistant. Fermentative on Oxidation Fermentation Media and hydrolyses Urea.

Escherichia coli.
Aerobic and facultatively anaerobic, gram negative bacilli which grows in ordinary media. Colonies are large, thick, grayish white, moist smooth opaque or partially translucent. It is hemolytic in blood agar. On MacConkey medium colonies are bright pink due to lactose fermentation. Indole and MR positive, VP and citrate utilization tests are negative. *E.coli* was the common gram negative bacilli in this study. *Escherichia coli* showed maximum sensitivity to Meropenem with 70.15%, Amikacin 82.35% ,Piperacillin- tazobactum 64.70%, Ciprofloxacin 35.29%. All the isolates showed resistance to Ampicilin.

Pseudomonas
Pseudomonas aeruginosa was the most common pathogen associated with non-diabetic foot ulcers in patients. It is aerobic gram negative motile bacilli, grows well on ordinary media producing large opaque irregular colonies with musky or earthy smell. The metabolism is oxidative and non-fermentative. Indole, MR-VP and H_2S tests are negative. Catalase,Oxidase and arginine dihydrolase tests are positive. In this study *Pseudomonas aeruginosa* was the most frequent one (50.34%). It has been reported that Imipenem is the most effective antibiotic against Gram-negative organisms, including *Pseudomonas aeruginosa*. In this study, 58.82% of the *Pseudomonas aeroginosa* isolates were sensistive to Imipenem and Meropenem. Additionally, we found that only 29.40% of *Pseudomonas aeruginosa* isolates were sensitive to gentamicin. Differences in the results obtained in many studies shows that the patterns of microbial infection are not consistent in patients therefore repeated evaluation of microbial characteristics and the antibiotic sensitivity is necessary for the selection of appropriate antibiotics.

Klebsiella pneumoniae.
Gram negative non-motile, non-sporing, capsulated coccobacilli. Large domeshaped mucoid colonies on routine media. Pink colonies on MacConkey agar. Catalase positive. Oxidase negative. Indole not produced, MR negative**,** VP positive**,** Citrate utilized, Urea hydrolysed, Decarboxylates lysine but not arginine and ornithine. *Klebsiella* isolates showed maximum sensitivity to Amikacin 90.90%, followed by Meropenem 72.72%. Gentamicin sensitivity is only 18.18%.

Acinetobacter
Gram negative non-motile coccobacilli. Non -haemolytic on bloodagar. Pale, non lactose fermenting colonieson MacConkey agar. Catalase positive, Oxidase negative, Nitrate not reduced to nitrite, Indole not produced, does not ferment sugars. Utilizes 10% lactose. *Acinetobacter* showed resistance to most of the antibiotic with maximum sensitivity to Meropenem 44.44%.

Proteus
Gram negative, pleomorphic motile bacilli. MR positive and VP negative. *Proteus* spp isolated were 100% sensitive to Imipenem.

CONCLUSIONS

It is always better to do culture and sensitivity before starting antibiotics inpatients with chronic ulcers. The most frequently isolated organisms like, *Pseudomonas, E. Coli, Klebsiella and, Acinetobacter* showed resistance to commonly used antibiotics.

Limitation of the study

This study is limited by its small size.

Acknowledgements

I thank Department of General Surgery and Department of Microbiology for allowing me to conduct this short study.

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